



TITLE:

STUDIES ON THE ANTITUBERCULOUS ACTIVITY OF ALPHA-ETHYL- THIOISONICOTINAMIDE- METHANESULFONATE AND ITS SIDE EFFECTS

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STUDIES ON THE ANTITUBERCULOUS ACTIVITY OF
ALPHA-ETHYL-THIOISONICOTINAMIDE-METHANESULFONATE
AND ITS SIDE EFFECTS

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INTRODUCTION

Alpha-ethyl-thioisonicotinamide (1314Th) was synthesized by D. Libermann et al.¹⁾, and has been found to be active against isoniazid (INH)-resistant, as well as INH-susceptible tubercle bacilli both *in vitro* and *in vivo*.

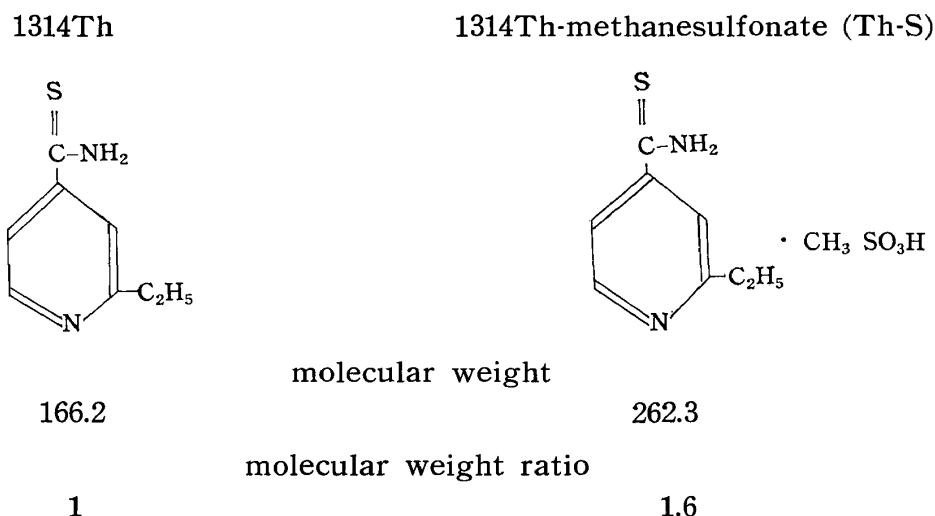
And it has been widely used in the treatment of tuberculous cases in whose sputa the tubercle bacilli had become resistant to streptomycin, PAS and INH.

The writers also have been interested in 1314Th and carried out several different investigations related to it²⁾³⁾. It was found that 1314Th was frequently badly tolerated by patients in poor condition, producing gastrointestinal symptoms (anorexia, nausea, gastralgia), renal and hepatic disturbances etc.

Many attempts have been made to modify the structure of the 1314Th to eliminate the side effects⁴⁾⁵⁾.

Alpha-ethyl-thioisonicotinamide-methanesulfonate (Th-S) is also one of the derivatives of 1314Th, synthesized in the Dainippon Pharmaceutical Co. Ltd., and a water-soluble tuberculostatic compound. The chemical structure and molecular weight of this compound are as follows:

Fig. 1



The writers were interested in Th-S and have done several fundamental and clinical trials with Th-S in order to study its antituberculous activity and its side effects.

EXPERIMENTS

MATERIALS AND METHODS

I. Activity *in vitro*

Drugs: Drugs tested were Th-S and 1314Th.

Medium: Kirchner's liquid medium with 10 per cent bovine serum was employed.

Inocula: Tenday-old culture of *Mycobacterium tuberculosis var. hominis* H37Rv strain in Tween-albumin liquid medium was diluted with the same culture medium to a concentration of about 1.0 mg. per ml., and agitated vigorously with a pipette in a test tube. After the large clumps of bacilli had settled, the supernatant of this initial suspension was diluted again with the same culture medium to the final concentration of 0.2 mg. per ml. The final bacillary suspension was dropped from Komagome's pipette into each test tube (12 by 110 mm.), and the inoculum size was 0.01 mg. per ml. of medium.

Experimental procedures: Twofold duplicate serial dilutions of each drug were made by successively transferring 2 ml. of Kirchner's medium. These serial tubes and control tubes were inoculated with tubercle bacilli (H37Rv strain). All tubes were incubated at 37C°. Four weeks later, all tubes were examined and the minimum inhibitory concentration (MIC) of each drug was recorded.

II. Activity *in vivo*

1. Acute toxicity of the Th-S in mice :

Male albino mice (dd-strain) were used for the experiment. They weighed 14 to 16 g. when they were received at the laboratory. The mice were housed in metal cages (not more than ten animals per cage) and were fed with standard mouse pellets and water.

2. Activity in mice :

Male albino mice (dd-strain) were used for the experiment. Infection was carried out by intravenous inoculation of 0.5 mg. of *Mycobacterium tuberculosis* var. *hominis* Kuroono strain.

Bacillary suspension was prepared from pellicles of 2 week-old cultures of the strain on the surface of glycerin bouillon. The initial suspension was diluted to a concentration of 5 mg. per ml. and the mice were inoculated with 0.1 ml. of the final suspension.

Drugs : Th-S, 1314Th, INH, viomycin (VM), pyrazinamide (PZA), sulfisoxazole (SI), cycloserine (CS), kanamycin (KM) and sodium *o*-aminophenol-methanesulfonate (SOM) were employed.

The administration of the drugs was started on the day following the intravenous inoculation of tubercle bacilli and was continued until half of the mice of any one of the treated groups were dead. The number of dead mice was plotted on a graph.

3. The tuberculostatic activity in the serum of humans after the administration of Th-S

The test doses of Th-S (400 mg. per person) and 1314Th (250 mg. per person, powder and enteric coated tablets) were administered orally. The blood samples for the test were collected aseptically before the drug-administration for the controls and 2, 4 and 6 hours after administration of the drugs and allowed to stand for 24 hours. Then the serum was separated from these samples by centrifugation, and modified Kirchner's medium added with serum to the concentration of 90 per cent were prepared by Shioda's method⁶⁾ for this experiment. The medium thus obtained is composed of 0.1 ml. of modified Kirchner's basic solution and 0.9 ml. of the serum. In the modified Kirchner's basic solution, all ingredients were added to 10 times the original concentration.

Inocula : Ten day-old culture of *Mycobacterium tuberculosis* H37Rv strain in Tween-albumin liquid media was diluted with saline solution to a concentration of about 0.5 mg. per ml. This suspension of bacilli was dropped

by Komagome's pipette into each test tube (12 by 110 mm.), and the inoculum size was 0.025 mg. per ml.

The components of modified Kirchner's basic solution are as follows:

Table 1.

disodium phosphate	3.0 g.
monopotassium phosphate	4.0 g.
magnesium sulphate	0.6 g.
sodium citrate	2.5 g.
asparagine	5.0 g.
glycerol	20.0 ml.
aq. dest.	add to 100.0 ml.
pH	6.2~6.4

RESULTS

I. Activity *in vitro*

Table 2 shows the tuberculostatic activity of 1314Th and Th-S. It was found that 1314Th and Th-S inhibited the growth of *Mycobacterium tuberculosis* H37Rv in Kirchner's medium with 10 per cent bovine serum in concentrations ranging from 0.625 to 1.25 γ per ml. The MIC of Th-S is somewhat higher than that of 1314Th, but as the molecular weight of Th-S was 1.6 times that of 1314Th, the tuberculostatic activity of Th-S was almost equal to that of 1314Th.

Table 2. Minimum Inhibitory Concentration of 1314Th and Th-S* (γ /ml.)

Material	Minimum inhibitory concentration
1314Th	0.625
Th-S	1.25

* Bacillary suspension was made from Tween-albumin culture of H37Rv strain.
Inoculum size was approximately 0.01 mg. per ml.

II. Activity *in vivo*

1. Acute toxicity of the Th-S in mice

The results are shown in table 3. It was found that the LD50 of Th-S was 1240 mg. per kg. when the compound was administered orally, 1000 mg. per kg. when the compound was injected subcutaneously, and 500 mg. per kg. when injected intravenously. It appears the acute toxicity of Th-S is some-

what less than that of 1314Th, but as the molecular weight of Th-S was 1.6 times that of 1314Th, the acute toxicity of Th-S showed almost equal to that of the 1314Th if this is taken into consideration.

Table 3. Acute Toxicity in Mice: LD50 (mg. per kg.)

Calculated by Litchfield and Wilcoxon's Method⁷⁾

Material	Oral	Subcutan.	Intravenous.
1314Th	850	—	—
Th-S	1240	1000	500

2. Activity in mice

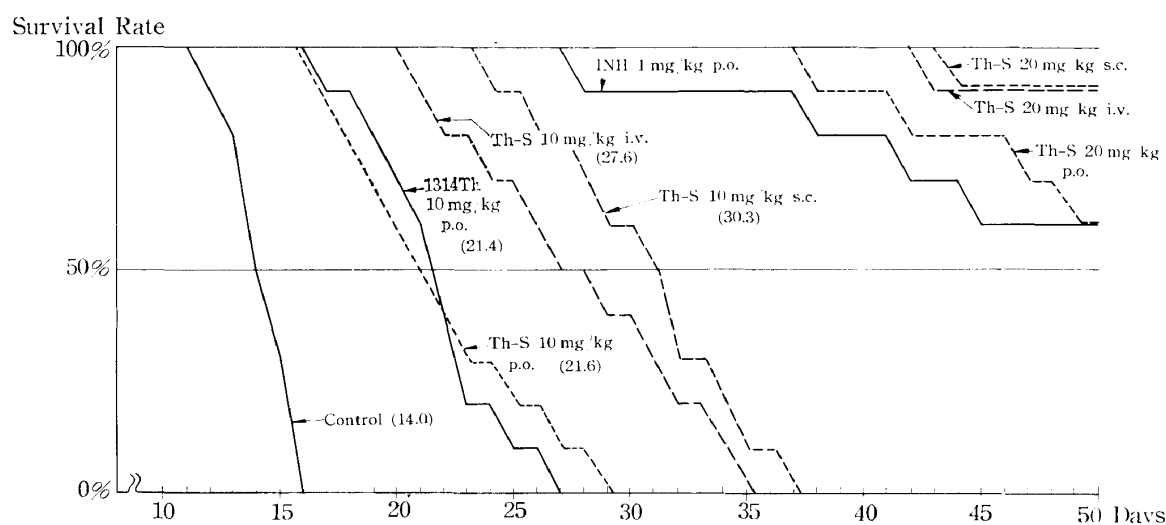
Influence of Th-S on the survival time (in days) of tuberculous mice was observed.

a. Single administration of Th-S

Ninety mice were randomly divided into nine groups of ten mice each and treated as follows:

- Group 1 Th-S 10 mg. per kg. daily, intravenously
- Group 2 Th-S 10 mg. per kg. daily, subcutaneously
- Group 3 Th-S 10 mg. per kg. daily, orally
- Group 4 Th-S 20 mg. per kg. daily, intravenously
- Group 5 Th-S 20 mg. per kg. daily, subcutaneously

Fig. 2. Survival Rate of Tuberculous Mice Treated with 1314Th and Th-S



Numerals in parenthesis indicate mean survival time in days.

- Group 6 Th-S 20 mg. per kg. daily, orally
- Group 7 INH 1 mg. per kg. daily, orally
- Group 8 1314Th 10 mg. per kg. daily, orally
- Group 9 Control (untreated)

Treatment was discontinued on the twenty-first day after the inoculation. The survival rates of these groups are shown in figure 2. Th-S 10 mg. per kg. orally showed the same therapeutic effect as 1314Th 10 mg. per kg. orally. Of the routes of drug-administration, subcutaneous injection was the most effective, next, intravenous injection, and oral administration was the least effective, but there was no significant difference among them.

b. Single administration of Th-S when the dose was decreased

Fifty mice were randomly divided into five groups of ten mice each and treated as follows:

- Group 1 1314Th 10 mg. per kg. daily, orally
- Group 2 1314Th 5 mg. per kg. daily, orally
- Group 3 Th-S 16 mg. per kg. daily, intravenously
- Group 4 Th-S 8 mg. per kg. daily, intravenously
- Group 5 Control (untreated)

Treatment was discontinued on the fourteenth day after the inoculation. The survival rates of these groups are shown in figure 3.

Th-S 16 mg. per kg. intravenously showed better therapeutic effect than that of 1314Th 10 mg. per kg. orally. But when the dose of Th-S was decreased, Th-S 8 mg. per kg. intravenously showed almost same therapeutic effect as 1314Th 5 mg. per kg. orally.

c. Combined administration of Th-S with other antituberculous drugs

Ninety mice were randomly divided into nine groups of ten mice each and treated as follows:

- Group 1 Th-S 16 mg. per kg. daily, intravenously
- Group 2 Th-S 8 mg. per kg. daily, intravenously
- Group 3 Th-S 8 mg. per kg. daily, intravenously and
VM 20 mg. per kg. twice a week, subcutaneously
- Group 4 Th-S 8 mg. per kg. daily, intravenously and
PZA 30 mg. per kg. daily, orally
- Group 5 Th-S 8 mg. per kg. daily, intravenously and
SI 30 mg. per kg. daily, orally

Fig. 3. Survival Rate of Tuberculous Mice Treated with ¹³¹I-Th and Th-S

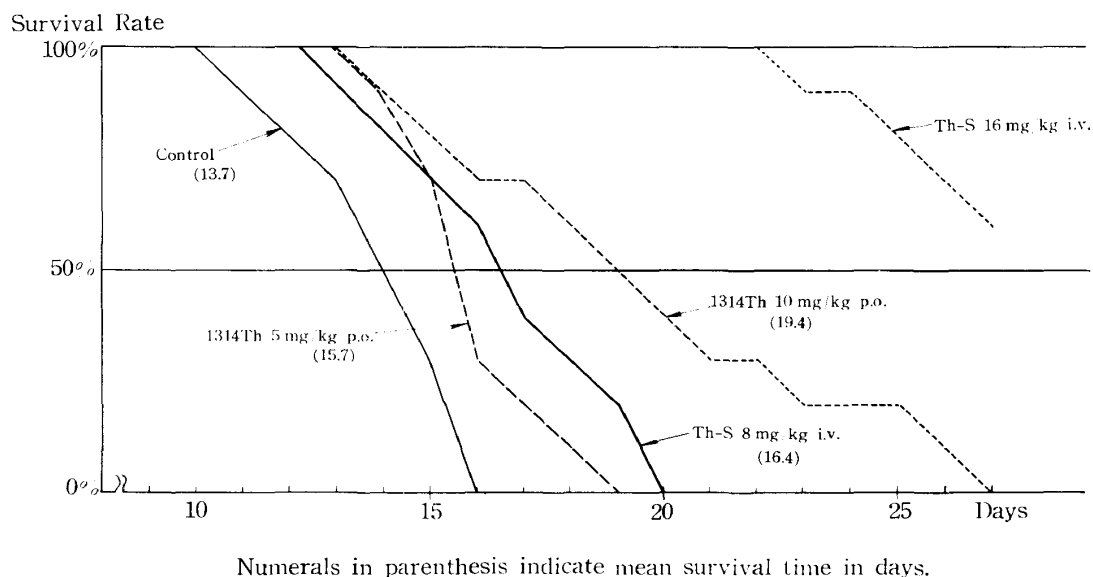
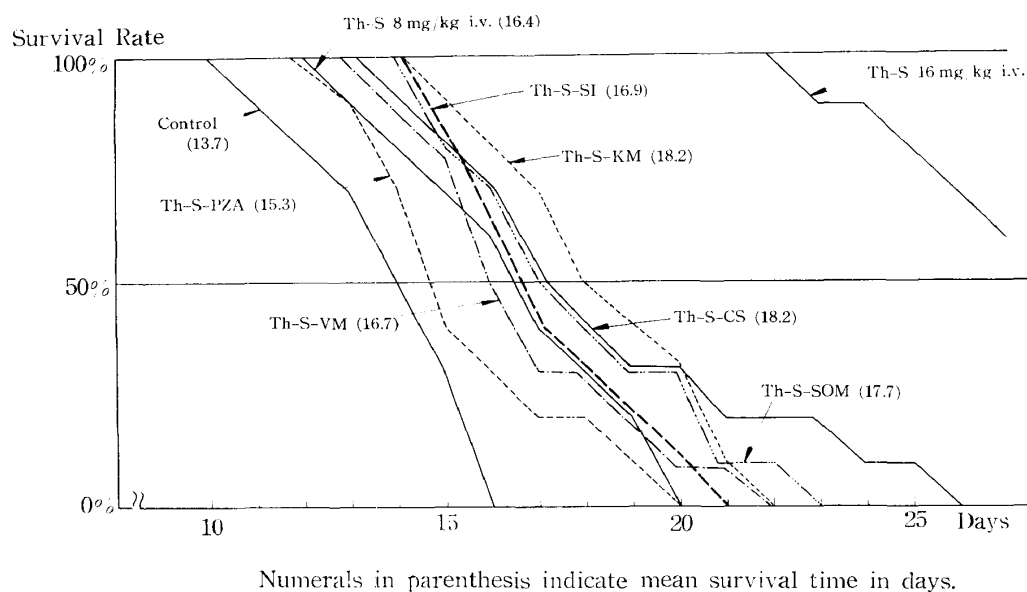


Fig. 4. Survival Rate of Tuberculous Mice Treated with Th-S and Other Antituberculous Drugs



Group 6	Th-S	8 mg. per kg. daily, intravenously and
	CS	5 mg. per kg. daily, orally
Group 7	Th-S	8 mg. per kg. daily, intravenously and
	KM	20 mg. per kg. twice a week, subcutaneously
Group 8	Th-S	8 mg. per kg. daily, intravenously and
	SOM	20 mg. per kg. daily, orally
Group 9	Control (untreated)	

Treatment was discontinued on the fifteenth day after the inoculation. Survival rates of these groups are shown in figure 4.

Th-S 16 mg. per kg. intravenously showed considerable effect. Then, in the experiment on the combined therapy the dose of Th-S was decreased to one half.

The combined treatment of Th-S with viomycin, pyrazinamide, sulfisoxazole, cycloserine, kanamycin and with o-aminophenol-methanesulfonate were compared with that of Th-S alone.

The treatment with Th-S 8 mg. per kg. and CS 5 mg. per kg., and with Th-S 8 mg. per kg. and KM 20 mg. per kg. were a little more effective than that with Th-S 8 mg. per kg. alone, but they were not as effective as Th-S 16 mg. per kg. alone.

3. The tuberculostatic activity in the serum of human after the administration of Th-S

Fig. 5. Duration in Hour of Bacteriostatic Activity of Serum after Administration of Drug

1314Th Enteric-Coated Tablets 250 mg.
(1 oral dose)

Time after administration of the drug.	Material				
	M.I	K.H	M.N	T.I	Y.F
Front					
2					
4					
6					

1314Th powder 250 mg.
(1 oral dose)

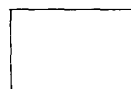
Time after administration of the drug.	Material				
	E.Y	Y.G	Y.S	E.H	T.S
Front					
2					
4					
6					

Th-S powder 400 mg.
(1 oral dose)

Time after administration of the drug.	Material				
	Y.H	T.N	S.H	K.K	S.K
Front					
2					
4					
6					



Tuberculostatic activity was
recognized.



Tuberculostatic activity was
not recognized.

As shown in Fig. 5, the tuberculostatic activity of the serum when Th-S was given orally was recognized up to 2 to 4 hours after administration, and this tuberculostatic activity seemed to appear earlier than that of 1314Th enteric coated tablet given orally.

CLINICAL TRIALS

As 1314Th was often poorly tolerated, producing gastrointestinal symptoms, the writers attempted intravenous drip of Th-S to the patients who poorly tolerated oral 1314Th. Th-S was dissolved in 300 ml. of physiological saline solution and was injected intravenously over about 3 hours.

The results were illustrated in table 4. The side effects (nausea, vomiting, headache, vertigo, etc.) were still fairly often, and of 43 patients, in 13 (34.9 per cent) the injection had to be discontinued within 1 month, 17 (39.5 per cent) within 2 months, 24 (55.8 per cent) within 3 months, 28 (65.1 per cent) within 4 months, and only 15 (34.9 per cent) tolerated the intravenous drip of Th-S well.

Thus the gastrointestinal side effects of 1314Th as well as Th-S were considered due to the injury after absorption.

The writers attempted to get the low but sustaining blood level of the drug. Of course the writers can not deny the possibility of the gastrointestinal side effects of 1314Th due to direct injury on stomach wall, but following their experience the enteric coated tablets of 1314Th were sometimes found in feces. So firstly 50 mg. of non enteric-coated 1314Th was administered orally 10 times a day and in the intolerated cases 25 mg. of 1314Th was administered 12 times a day.

The results were illustrated in table 5. In this case, side effects were relatively few, and of 112 patients, in only 33 (29.5 per cent) the administration had to be discontinued within 4 months.

Then, the writers attempted the divided administration of Th-S to the patients who had to be discontinued the administration of 1314Th.

800 mg. (daily dose) of Th-S corresponds to 500 mg. of 1314Th, and 500 mg. of Th-S corresponds to 300 mg. of 1314Th. 80 mg. or 50 mg. (in the intolerated cases) of Th-S was administered orally ten times a day.

The results were illustrated in table 6. And of the 24 patients, in 17 (70.8 per cent) the administration was well tolerated for 4 months.

Table 4. Side Effects of Intravenous Drip of Th-S in 43 Patients

Total Cases	43	
Periods	Number of intolerated cases	(per cent)
Within first month	15	(34.9)
Within second month	17	(39.5)
Within third month	24	(55.8)
Within fourth month	28	(65.1)
Well tolerated cases	15	(34.9)

Table 5. Side Effects of 50 mg. of 1314Th (Non Enteric-Coated) Administered 10 Times a Day or at least 25 mg. of 1314Th Administered 12 Times a Day

Total Cases	112	
Intolerated Cases	33	(29.5)
Well Tolerated Cases	79	(70.5)

Table 6. Side Effects of 80 mg. or 50 mg. of Th-S (Taking into Consideration of Molecular Weight) Administered 10 Times a Day

Total Cases	24	
Intolerated Cases	7	(29.2)
Well Tolerated Cases	17	(70.8)

DISCUSSION

The present investigations show that Th-S has a certain antituberculous activity.

The writers observed that the minimum inhibitory concentration of Th-S against the strain (H37Rv) in Kirchner's liquid medium containing 10 per cent bovine serum proved to 1.25 γ per ml. The MIC of TH-S is somewhat higher than that of 1314Th, but if the molecular weight of Th-S is taken into consideration, the tuberculostatic activity of Th-S is almost equal to 1314Th. The acute toxicity in mice and activity in mice were investigated, and LD50 was found to be 1240 mg. per kg. when the compound was administered orally, 1000 mg. per kg. when injected subcutaneously, and 500 mg. per kg. by intravenous injection. And if the molecular weight of Th-S was taken into consideration, there was no significant difference found between 1314Th and Th-S. In the experiments on mice, the therapeutic effect of Th-S showed the same good therapeutic effect of 1314Th.

The tuberculostatic activity in the human serum after administration of Th-S was investigated. This tuberculostatic activity was seemed to appear a little earlier than that of enteric-coated 1314Th given orally.

Up to this point, there is no significant difference in antituberculous activity between 1314Th and Th-S. Then the intravenous drip of Th-S was tried and found to be ineffective to decrease the side effects. But the divided administration of non enteric-coated 1314Th was investigated with good results. At last the best results were obtained in the use of divided administration of Th-S. But the influence of the divided administration of 1314Th or Th-S on their therapeutic effects has not been settled yet. This problem is now being examined in animals.

SUMMARY

Observations were presented on the activity *in vitro* and *in vivo* of alpha-ethyl-thioisonicotinamide-methanesulfonate (Th-S). This compound is a water-soluble substance. The molecular weight of Th-S is 262.3, 1.6 times that of 1314Th (166.2).

1. The minimum inhibitory concentration (MIC) of Th-S against H37Rv strain in Kirchner's liquid medium with 10 per cent bovine serum, proved to be 1.25 γ per ml.

2. The toxicity of Th-S in mice was tested, and LD50 was found to be 1240 mg. per kg. when the compound was administered orally, 1000 mg. per kg. when injected subcutaneously, and 500 mg. per kg. by intravenous injection.

3. Mice infected with tubercle bacilli of human origin (Kuroono strain) were employed. In this experiment, the therapeutic effect of Th-S showed the same good therapeutic effect of 1314Th.

4. The tuberculostatic activity of the serum when Th-S was given orally was recognized up to 2 to 4 hours after administration, and this tuberculostatic activity seemed to appear earlier than that of 1314Th enteric-coated tablet given orally.

5. The writers attempted intravenous drip of Th-S to patients poorly tolerating 1314Th. The side effects were still fairly often. Divided oral administration of Th-S was better tolerated than that of 1314Th by these patients.

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